

Appendix B

1. (Amended) A method for measuring the ability of a compound to affect the binding activity of molecules to a steroid hormone receptor~~receptors~~, comprising:

- a. ~~mixing~~providing a solution comprising a fluorescence-emitting compound that binds to the steroid hormone ~~receptors~~ receptor at a first domain; and a fluorescence-labeled nucleic acid that binds to the steroid hormone ~~receptors~~ receptor at a second domain; and in a solution containing the steroid hormone ~~receptors~~ receptor;
- b. measuring the fluorescence polarization of each fluorescence emission from said fluorescence-emitting compound and said fluorescence-labeled nucleic acid present in the solution from step a) at excitation and emission wavelengths corresponding to the excitation and emission wavelengths of each of said fluorescence-emitting compound and said fluorescence-labeled nucleic acid;
- c. incubating the solution of step a) with at least one ~~molecule~~ unlabeled compound that may compete for interaction with~~affect the binding of said fluorescence-emitting compound or said fluorescence-labeled nucleic acid to said steroid hormone receptor at least one domain;~~
- d. measuring the fluorescence polarization of each fluorescence emission of said fluorescence-emitting compound and said fluorescence-labeled nucleic acid present in the solution during from step c) at excitation and emission wavelengths corresponding to the excitation and emission wavelengths of each of said fluorescence-emitting compound and said fluorescence-labeled nucleic acid; and,

A

e. comparing the fluorescence polarization measurements of step b) with step d) to determine if said unlabeled compound affects the binding of said fluorescence-emitting compound or said fluorescence-labeled nucleic acid to the steroid hormone receptor~~quantify any interaction.~~

2. (Amended) The method of claim 1 wherein the steroid hormone ~~receptors are~~receptor is purified.

3. (Amended) The method of claim 2 wherein the purified steroid hormone ~~receptors comprises~~is a recombinant steroid hormone receptor.

4. (Amended) The method of claim 2 wherein the difference in fluorescence polarization between the bound and unbound fluorescence-emitting compound and between the bound and unbound fluorescence-labeled nucleic acid~~quantitation comparison of step e)~~ is of sufficient magnitude to be suitable for use with a screening assay.

7. (Amended) The method of claim 3 wherein the steroid hormone ~~receptors~~receptor comprises estrogen receptor.

